DEPARTMENT OF THE AIR FORCE
AIR FORCE RESEARCH LABORATORY
WRIGHT-PATTERSON AIR FORCE BASE OHIO 45433

28 June 00

MEMORANDUM FOR US EPA
NCEA (MD-52)
RTP, NC 27711
ATTN: ANNIE M. JARABEK

FROM: Kyung O. Yu, Ph.D.
AFRL/HEST
Operational Toxicology Branch
2856 G Street, Bldg 79
Wright-Patterson AFB OH 45433-7400

SUBJECT: Consultative Letter, AFRL-HE-WP-CL-2000-0038, Tissue Distribution and Inhibition of Iodide Uptake in the Thyroid by Perchlorate with Corresponding Hormonal Changes in Pregnant and Lactating Rats (drinking water study).

1. The Operational Toxicology Branch conducted pharmacodynamic studies on perchlorate tissue distribution, perchlorate induced inhibition of iodide uptake and corresponding hormone changes in pregnant and lactating Sprague-Dawley female rats. Perchlorate (0, 0.01, 0.1, 1.0 or 10.0 mg/kg-day) was administered in the drinking water from gestation day (GD) 2 through GD20, postnatal day (PND) 5 or PND10. Thyroid stimulating hormone, free and bound thyroxine and triiodothyronine were measured in the serum of the dams, fetuses and pups. Perchlorate concentrations were measured in the serum, thyroid, mammary gland, skin and gut contents of the dams, fetuses and pups as well as in the dams' milk on PND10. The attached report summarizes the data collected and analyzed to date.

2. These studies are currently being used in development of a physiologically based pharmacokinetic (PBPK) model for perchlorate in the lactating rat. The lactation model describes perchlorate dosimetry in the thyroid, serum and milk of the lactating dams. Data from on-going and future animal investigations will augment the pregnancy and lactation PBPK modeling efforts.

3. If you have any questions about this letter, please contact me by phone: 937-255-5150 ext. 3184.

Kyung O Yu

KYUNG O YU, Ph.D.
Operational Toxicology Branch

Attachment: Tissue Distribution and Inhibition of Iodide Uptake in the Thyroid by Perchlorate with Corresponding Hormonal Changes in Pregnant and Lactating Rats (drinking water study) Report

1st Ind, AFRL/HEST 28 June 2000

MEMORANDUM FOR US EPA
ATTN: MS. ANNIE JARABEK

This letter report has been coordinated at the branch level and is approved for release.

David R. Mattie

DAVID R. MATTIE, Ph.D.
Acting Chief
Operational Toxicology Branch
Human Effectiveness Directorate

Tissue Distribution and Inhibition of Iodide Uptake in the Thyroid by Perchlorate with Corresponding Hormonal Changes in Pregnant and Lactating Rats (drinking water study)

Kyung O. Yu, Ph.D.[1], Deirdre A. Mahle[2], Latha Narayanan[3], Richard J. Godfrey[2], Gerry W. Buttler[2], Paula N. Todd[1], Margaret A. Parish[2], James D. McCafferty[1], Todd A. Ligman[1], Charles D. Goodyear[4], Teresa R. Sterner[5], Timothy A. Bausman[1], David R. Mattie, Ph.D.[1] and Jeffrey W. Fisher, Ph.D.[1]

[1]AFRL/HEST
2856 G St, Bldg 79
Wright-Patterson AFB, OH 45433

[2]ManTech Environmental Technologies, Inc.
P.O. Box 31009
Dayton, OH 45437-0009

[3]GEOCENTERS, Inc.
2856 G St, Bldg 79
Wright-Patterson AFB, OH 45433-7400

[4]StatisticalConsultant
Waynesville, OH 45068

[5]Operational Technologies Corporation
1370 N. Fairfield Rd, Ste. A
Dayton, OH 45432

28 June 2000

INTRODUCTION

Ammonium perchlorate is a powerful oxidizer used in solid rocket fuel mixtures. Perchlorate salts are also present in fireworks, air bag inflators and commercial fertilizers. Perchlorate anions (ClO_4^-) are formed by the dissociation of perchlorate salts. Perchlorate has been found to contaminate soil, groundwater and surface water in more than 11 states (Urbansky, 1998; Urbansky and Shock, 1999). This contamination has resulted in concern over the potential health effects of long term ingestion of perchlorate in drinking water, particularly in infants and children (Mattie and Jarabek, 1999).

The perchlorate ion is similar in size to that of inorganic iodide. This allows perchlorate to be competitively taken up by the sodium iodide symporter in the thyroid, thereby reducing the amount of iodide available in the thyroid for thyroid hormone production (Wolff, 1998). Thyroid hormones are essential to physical and mental development during the first two years of human life (Bakke *et al.*, 1976; Porterfield, 1994). Several tissues show sodium iodide symporter activity in addition to the thyroid, and may be susceptible to perchlorate induced iodide uptake inhibition. These tissues include the mammary gland, salivary gland, placenta, skin, ovary and gastrointestinal tract (Brown-Grant, 1961).

The objective of these studies was to determine the perchlorate distribution in maternal, fetal and pup tissues of Sprague-Dawley rats and the corresponding hormonal changes in both the dams and offspring. Perchlorate inhibition of iodide uptake in maternal thyroids was also determined. These studies were performed with the intent to develop physiologically based pharmacokinetic (PBPK) models for perchlorate in the pregnant and lactating rat.

METHODS

Timed pregnant Sprague-Dawley rats were provided perchlorate in drinking water from receipt on gestation day (GD) 2 until scheduled euthanasia. GD0 was designated as the day the vaginal plug was formed and postnatal day (PND) 1 was the day of birth. At birth, pups were culled to eight per litter (four males and four females) for the PND10 group.

Euthanization of rats by CO_2 asphyxiation was always scheduled in the morning (5:30 am through 11:30 am) to minimize the influence of diurnal variation on thyroid hormone levels in serum (Pallaedo *et al.*, 1976). Serum was obtained after centrifugation of blood at 3000 rpm for 15 minutes.

Methods for analysis of perchlorate and ^{125}I levels in tissues and serum levels of thyroid stimulating hormone (TSH), thyroxine (T_4), free T_4 and triiodothyronine (T_3) are reported elsewhere (Yu *et al.*, 2000). Carrier-free ^{125}I has a specific activity of 16.0 mCi/μg.

Inhibition of ^{125}I Uptake

Pregnant and lactating female rats (n=6) were provided drinking water containing perchlorate with 1 of 5 target doses (0, 0.01, 0.1, 1.0 and 10.0 mg/kg-day) from GD2 until either GD20 or PND5. The GD20 group was challenged with an oral dose of 33 µg/kg and the PND5 group with 39.6 µg/kg of non-radiolabeled iodide and carrier-free ^{125}I mixed in physiological saline. Rats were euthanized at two hours (h) post dosing. The following tissues were collected and analyzed for perchlorate and ^{125}I concentrations.

- GD20 dams: blood, thyroid, skin, amniotic fluid, placenta, mammary gland, gastric tract and gastrointestinal (GI) contents
- GD20 fetuses: pooled blood and gastric tract from all fetuses of each dam; skin of each fetus collected separately
- PND5 dams: blood, thyroid, skin, mammary gland and GI contents
- PND5 pups: blood of male and female pups pooled by sex; skin, gastric tract and GI contents of each pup collected separately

Thyroid Hormones

Pregnant and lactating female rats (n=8) were provided drinking water containing perchlorate with 1 of 5 target doses (0, 0.01, 0.1, 1.0 and 10 mg/kg-day) from GD2 until either GD20 or PND5. At the end of drinking water exposures (GD20 or PND5), rats were euthanized. The following tissues were collected for analysis of perchlorate. Serum levels of TSH, T_3 and T_4 were also measured.

- GD20 dams: blood, thyroid, amniotic fluid, mammary gland, skin, placenta and GI contents
- GD20 fetuses: pooled blood and gastric tract from all fetuses of each dam; skin of each fetus collected separately
- PND5 dams: blood, thyroid, skin, mammary gland and GI contents
- PND5 pups: blood of male and female pups pooled by sex; skin, gastric tract and GI contents of each pup collected separately

Milk Transfer

Dams were provided drinking water containing perchlorate with 1 of 5 target doses (0, 0.01, 0.1, 1.0 and 10 mg/kg-day) from GD2 until PND10 (n=6 dams per group and n=8 pups per litter). To collect milk, dams were administered a 0.6 mL/kg intraperitoneal injection of a mixture containing ketamine (42 mg/kg), xylazine (3.6 mg/kg) and oxytocin (0.25 mL/kg, 1.0 mL equals 10 USP unit). Within 10 minutes, milk was expressed into capillary tubes by manually massaging the mammary tissue near the nipple (Fisher *et al.*, 1990). Once milk was expressed, the dam was euthanized and tissues were collected. While the dam was prepared for milk collection, litters were euthanized to harvest tissues. The following tissues were collected for perchlorate analysis.

- PND10 dams: milk, blood, thyroid, skin, mammary gland, gastric tract and GI contents

- PND10 pups: blood of male and female pups pooled by sex; skin, gastric tract and GI contents of each pup collected separately

Statistical Analysis

A one-way analysis of variance was performed to determine whether there was a significant difference among dose groups. All paired tests among groups used two-tailed two-sample t-tests. All statistical comparisons utilized a minimum significance level of $p<0.05$.

RESULTS

Pregnant rats were treated with perchlorate (0, 0.01, 0.1, 1.0 and 10.0 mg/kg-day) in drinking water during gestation. The rats were challenged with an oral bolus of ^{125}I on GD20 and killed two hours later. Table 1 displays the actual dose rates for perchlorate by measuring perchlorate in the drinking water solution, body weight and daily water consumption. Figure 1 displays the perchlorate levels in GD20 maternal and fetal serum, amniotic fluid, dam skin and GI content. Perchlorate concentration was higher in the skin, thyroid (Table 2) and GI content of the dam compared to the dam serum (Figure 1). Perchlorate concentration in fetal serum was lower than maternal concentration, suggesting that the placenta acts as a diffusion-limited barrier for transfer of perchlorate to the fetus. The rapid increase in serum and tissue perchlorate concentrations at the 10 mg/kg-day dose resulted in a "hockey-stick" shaped perchlorate concentration profile (Figure 1 and Table 2). This probably reflects a transition from flow-limited to capacity-limited systemic clearance of perchlorate.

TABLE 1. NOMINAL AND CALCULATED DOSE RATES BASED ON MEASURING PERCHLORATE IN DRINKING WATER CONSUMPTION AND BODY WEIGHT

Nominal dose mg/kg-day	Measured dose (mg/kg-day) GD20	PND5	PND10
0.01	0.012	0.012	0.012
0.1	0.08	0.114	0.18
1	1.08	1.11	1.16
10	10.90	11.47	11.37

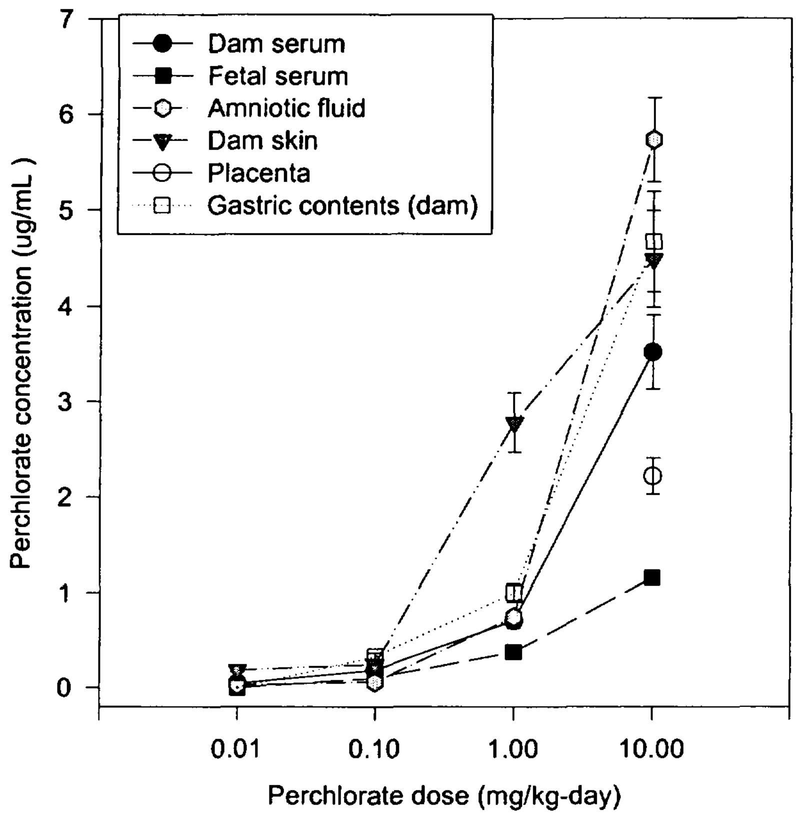

Figure 1. Perchlorate concentrations in tissues of dams and fetuses (GD20). Pregnant rats were provided with perchlorate in drinking water during gestation. Data are mean ± SD (n=6).

TABLE 2. PERCHLORATE CONCENTRATIONS (μg/g) IN THE THYROID OF PREGNANT AND LACTATING FEMALE RATS PROVIDED PERCHLORATE IN DRINKING WATER DURING GESTATION AND LACTATION. DATA ARE MEAN ± STANDARD DEVIATION (SD) (n=6).

	0.01 mg/kg-day	0.1 mg/kg-day	1 mg/kg-day	10 mg/kg-day
GD20	1.08 ±0.13	5.99 ± 0.73	27.63 ± 2.65	70.32 ± 7.21
PND5	0.70 ± 0.09	4.69 ± 0.49	14.29 ± 1.76	61.22 ± 6.61
PND10	1.13 ± 0.15	4.45 ± 0.52	22.29 ± 0.52	43.86 ± 4.15

Two hours after an oral bolus dose with ^{125}I, a substantial (and somewhat variable) amount of ^{125}I was found in the GI content of the dam. Studies were not performed to examine systemic uptake of ^{125}I after oral bolus administration. However, the residual ^{125}I radioactivity in the GI contents is thought to be from active uptake of ^{125}I from systemic circulation into the gut. The ^{125}I concentration in the dam GI contents diminished with increasing dose of perchlorate, while the serum ^{125}I concentrations rose slightly with increasing dose of perchlorate (Figure 2A). Fetal serum concentration of ^{125}I was below maternal concentration of ^{125}I, suggesting that the placenta acts as a diffusion limited barrier (Figures 2A and B). Perchlorate did not appear to influence the uptake of ^{125}I in the placenta and diffusion across the placenta into fetal circulation. The uptake of total iodine in the fetal GI tract was substantial and variable. The gastric tract in the GD20 fetus actively sequesters ^{125}I. These data are consistent with other reports on gastric tract sequestration of iodide (Brown-Grant, 1961). Total iodine concentrations in dam skin, placenta and mammary gland were below maternal serum total iodine concentrations (data not shown).

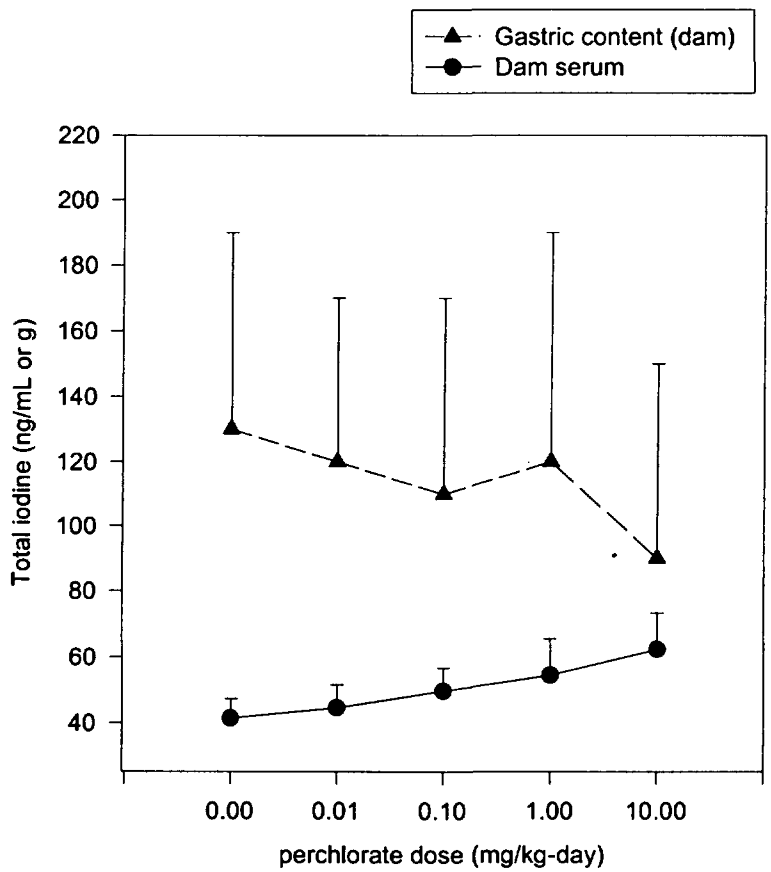

Figure 2A. Total iodine levels in tissues of dams (GD20). Pregnant rats were provided perchlorate in drinking water. After 18 days of treatment with perchlorate, pregnant rats were challenged with ^{125}I. Data are mean ± SD (n=6).

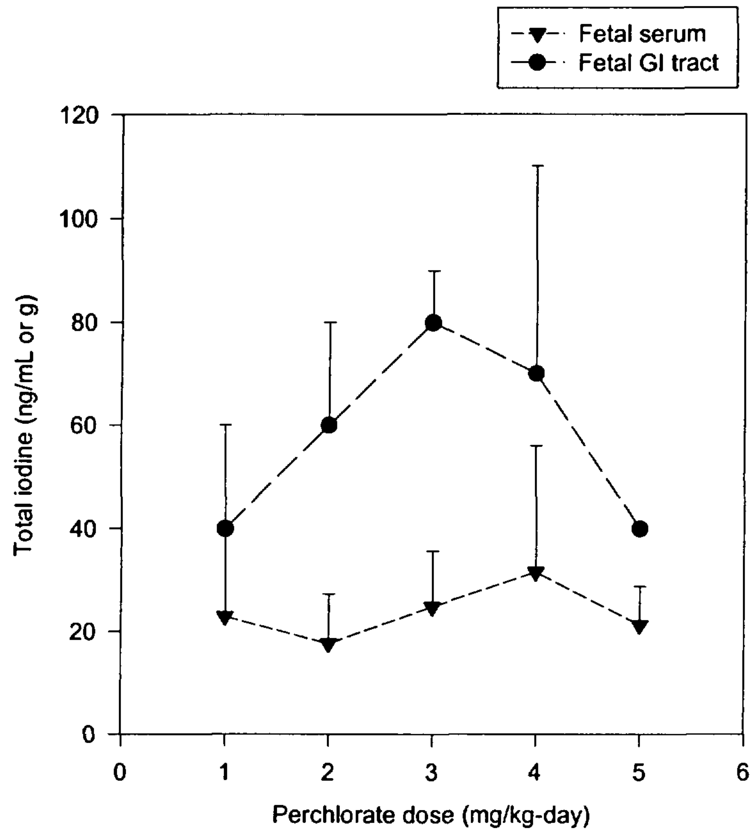

Figure 2B. Total iodine levels in tissues of fetuses (GD20). Pregnant rats were provided perchlorate in drinking water. After 18 days of treatment with perchlorate, pregnant rats were challenged with ^{125}I. Data are mean ± SD (n=6).

Uptake of ^{125}I into the maternal thyroid was variable (Figure 2C), with an apparent trend of decreasing concentration of bound iodine in the thyroid. The maternal thyroid appears to compensate for the inhibitory effects of perchlorate by dose-dependent up-regulation of serum TSH (Figure 3). Serum TSH levels also increased in a dose-dependent manner in the GD20 fetus. Both T_4 and T_3 serum levels decreased in a dose-dependent manner in the GD20 dam and fetus, although statistical significance varied (Figure 3). In both the dam and fetus, free T_4 concentrations in the serum increased in a dose-dependent manner.

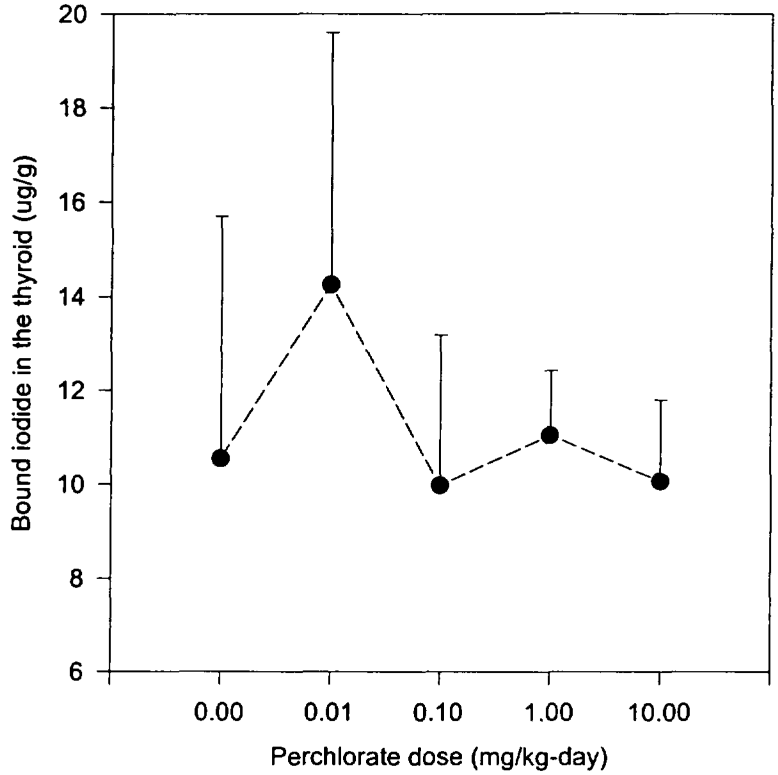

Figure 2C. Bound iodide in the thyroid of GD20 dam. Pregnant rats were provided perchlorate in drinking water. After 18 days of treatment with perchlorate, pregnant rats were challenged with ^{125}I. Data are mean ± SD (n=6).

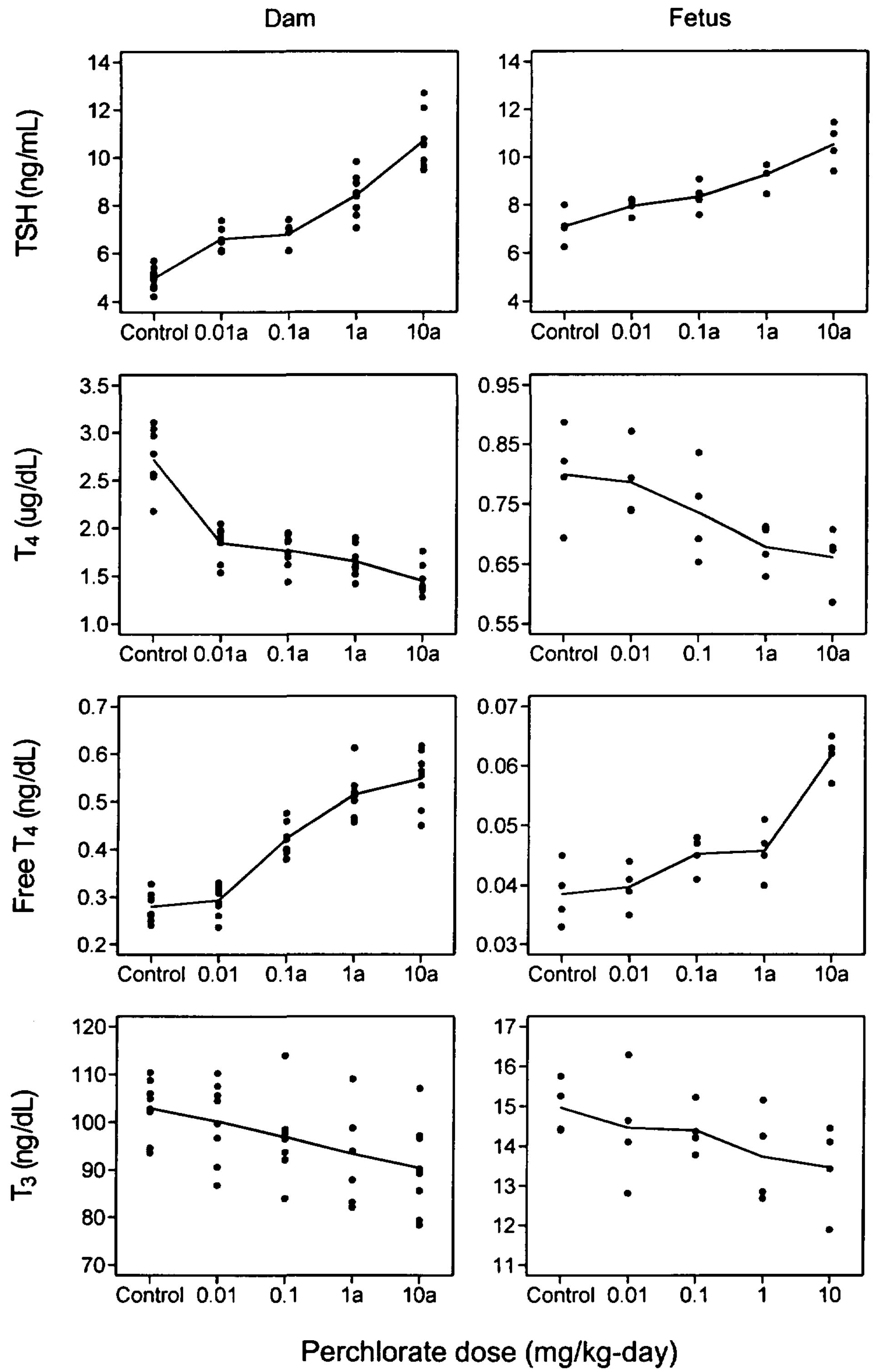

Figure 3. Serum TSH and thyroid hormone values for the GD20 dams and fetuses. Pregnant rats were treated with perchlorate in drinking water. Line segments connect mean from each dose group (dam n=6; pooled fetus n=4). Comparisons with control (a = p<0.05) used two-tailed t-test with pooled error.

Perchlorate dosimetry in the lactating dam and nursing pup (Figure 4A) was similar to that in the GD20 pregnant dam. Perchlorate was sequestered into the GI contents, skin (Figure 4A) and thyroid (Table 2). The mammary gland (only analyzed at 10 mg/kg-day) did not appear to sequester perchlorate on PND5 (Figure 4A), as suggested by the lower concentration in the gland as compared to maternal serum. The tissue and serum perchlorate concentrations from the 10 mg/kg-day dose group were high, relative to the other dose groups for both the pups and dams. Serum perchlorate concentrations were similar between male and female pups. Serum perchlorate concentrations in the male and female pups also were similar to those in the dam, with the exception of the 10 mg/kg-day dose group. This suggests that systemic clearance of perchlorate in the young pup is probably much slower than in the dam.

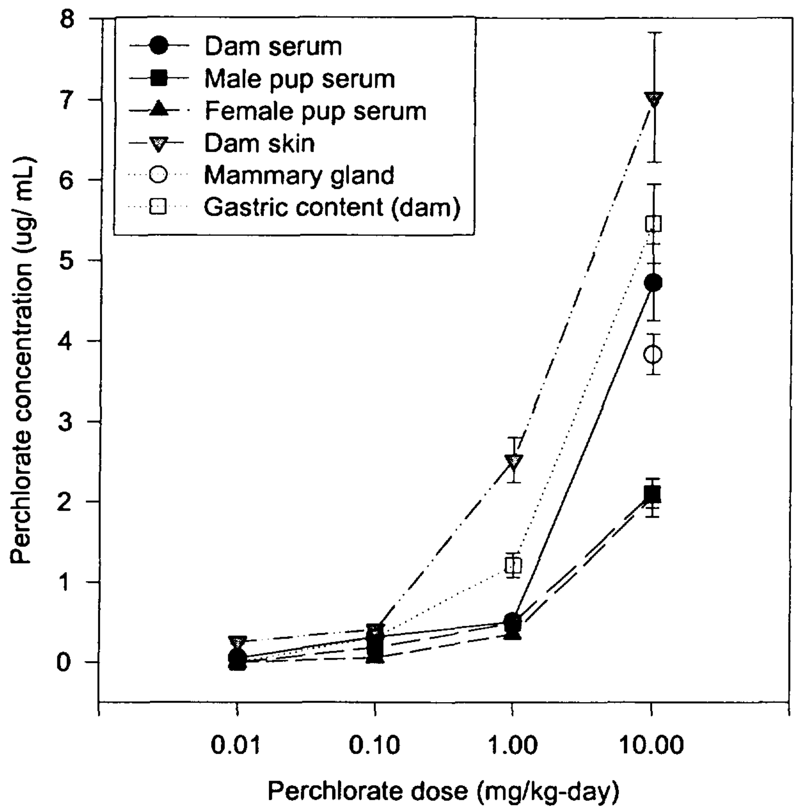

Figure 4A. Female rats were treated with perchlorate in drinking water for 19 days of gestation and for 5 days of lactating period. Perchorate concentrations were determined in tissues of dams and pups. Data are mean ± SD (n=6).

In a separate study, lactational transfer of perchlorate was measured by milking PND10 rats given drinking water containing perchlorate throughout gestation and lactation. Perchlorate levels in milk were higher than in maternal serum (Figure 4B), suggesting that perchlorate is actively sequestered into milk by PND10. Male and female pup serum levels of perchlorate were similar to the dam.

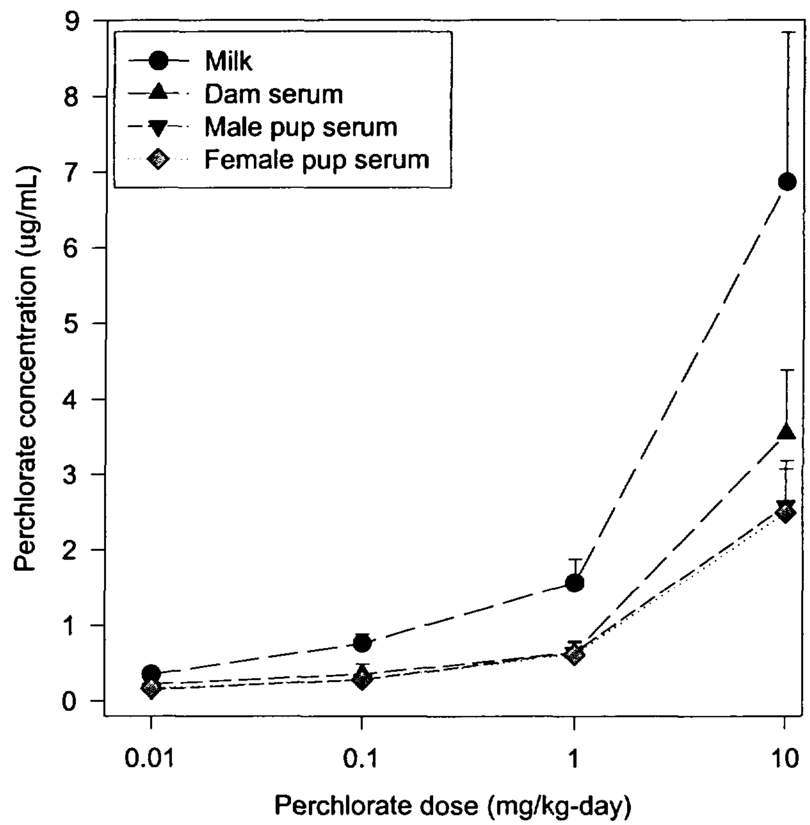

Figure 4B. Perchlorate concentrations in tissues of pups and dams dosed with perchlorate in drinking water (PND10). Data are mean ± SD (n=6).

Two hours after an oral bolus dose of ^{125}I in PND5 nursing dams, ^{125}I radioactivity in mammary gland, although variable, was higher than that in maternal serum (Figure 5A), demonstrating active uptake of ^{125}I in the mammary gland. ^{125}I was also detected in the GI contents of the lactating dam. No time course studies on oral uptake of ^{125}I were performed in the lactating rat. ^{125}I concentrations in male and female pup serum (Figure 5B) do not appear to be significantly affected by perchlorate exposure at or below 10 mg/kg-day, although there was a dose-dependent trend of decreased ^{125}I in the mammary tissue (Figure 5A). Bound iodine concentrations in the thyroid (Figure 5C) suggest that the thyroid was up-regulated by TSH because the perchlorate treatment group levels were similar to control values. Hormone analyses of PND5 dams verified this observation (Figure 6), showing dose-dependent increases in serum TSH and decreased T_4 and T_3 serum levels. The effect of perchlorate on the hormone profile on the male and female rat pups is less clear and may indicate the possible existence of sex differences (Figure 6).

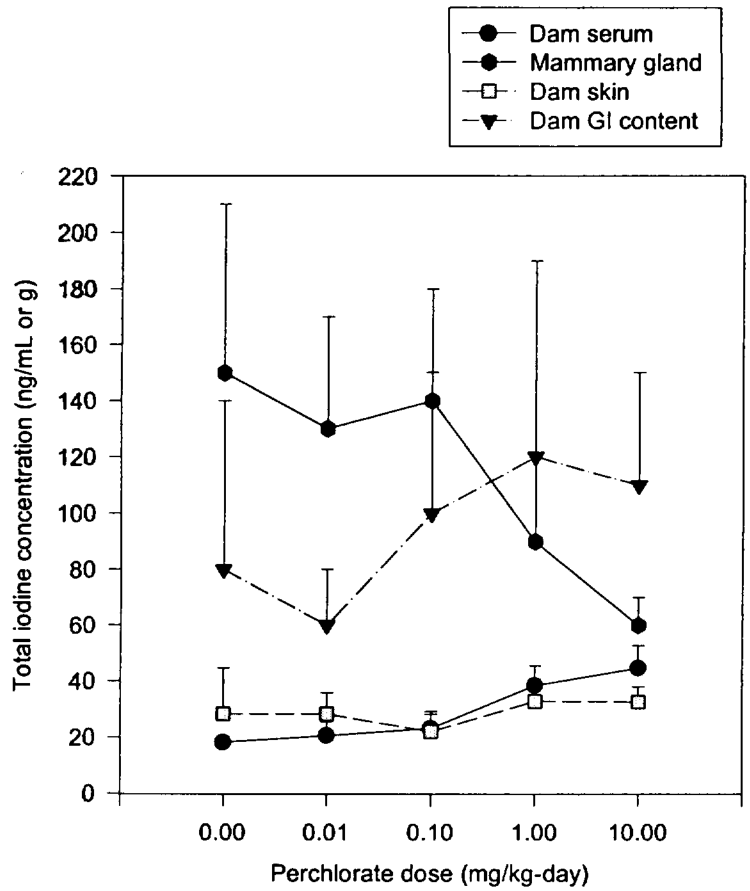

Figure 5A. Total iodine concentartions in tissues of dams (PND5). Lactating rats were treated with perchlorate in drinking water then challenged with ^{125}I. Data are mean ± SD (n=6).

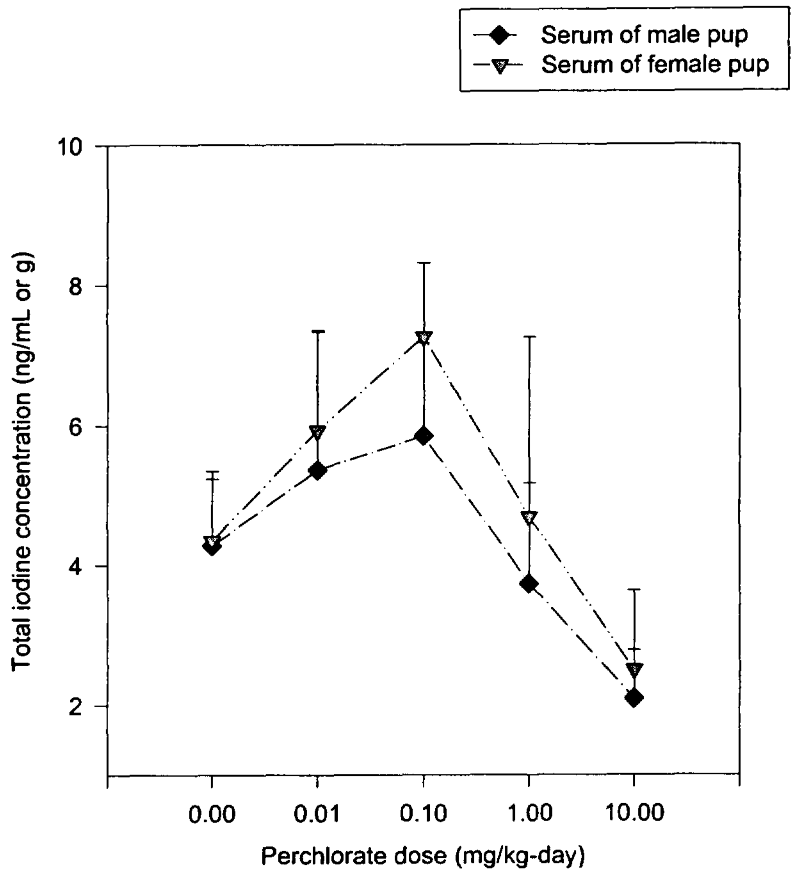

Figure 5B. Total iodine concentrations in serum of male & female pups (PND5). Lactating rats were treated with perchlorate in drinking water then challenged with ^{125}I. Data are mean ± SD (n=6).

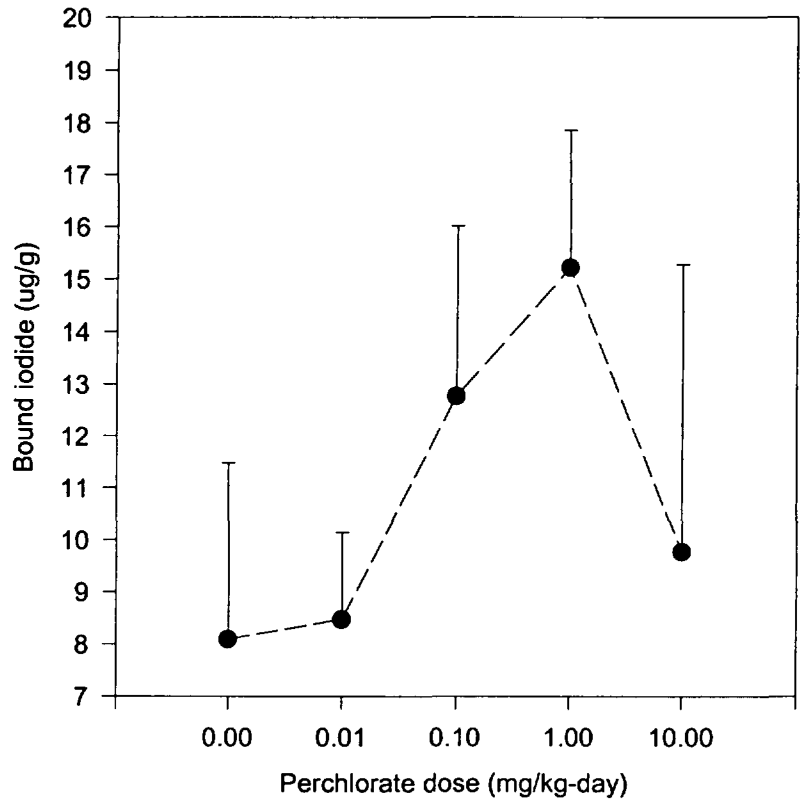

Figure 5C. Bound iodide in the thyroid of PND5 dams. Pregnant rats were provided perchlorate in drinking water. Data are mean ± SD (n=6).

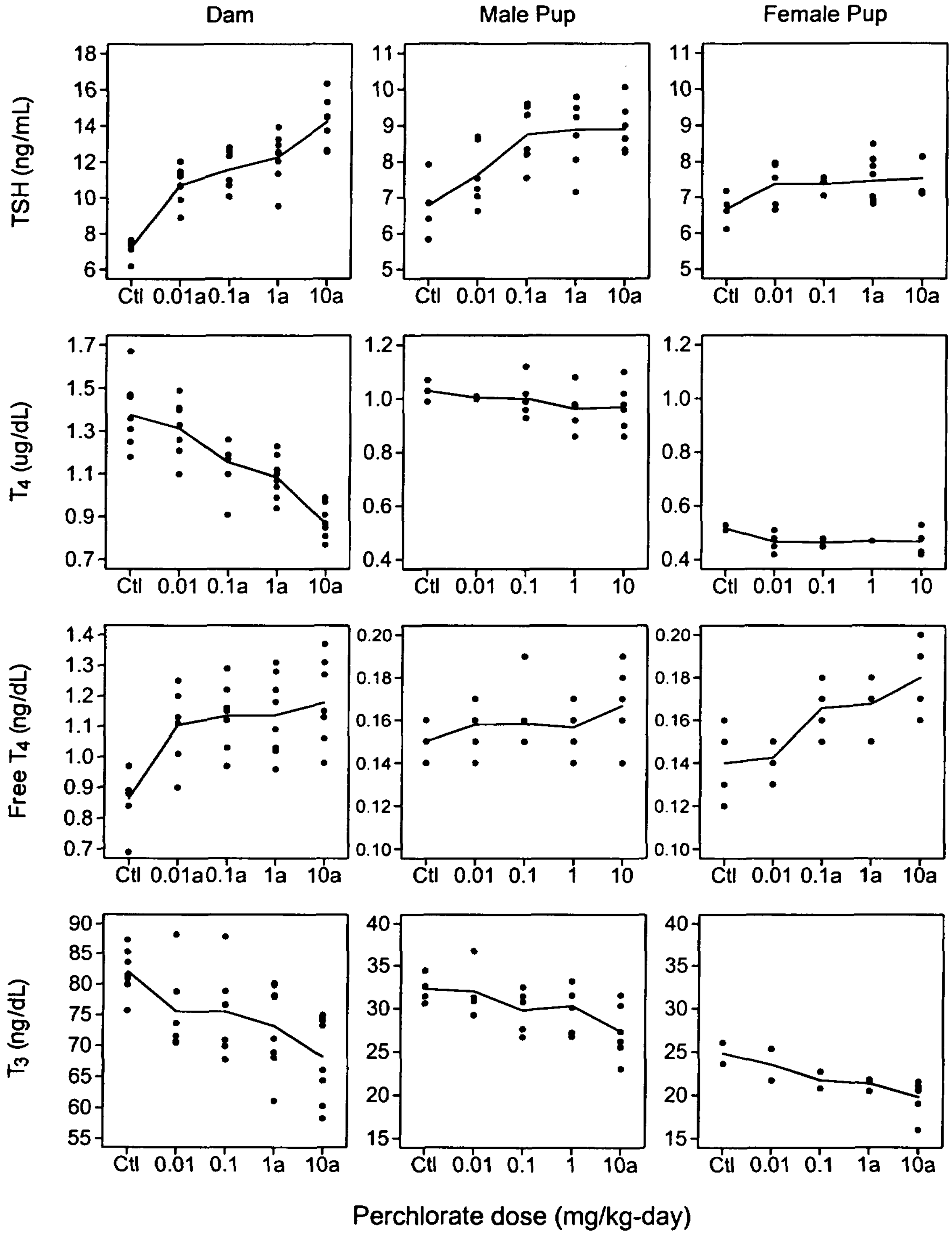

Figure 6. TSH and thyroid hormone values for the PND5 dams and male and female pups. Lactating rats were treated with perchlorate in drinking water. Line segments connect mean from each dose group (dam n=6; pooled male and female pups n=4). Comparisons with control (a = p<0.05) used 2-tailed t-test with pooled error.

SUMMARY

Perchlorate concentrations in skin and GI contents of the dams at GD20 and PND5 and in milk at PND10 were higher than the serum concentrations, indicating active perchlorate sequestration in these tissues. Dose-related increases of perchlorate concentrations in the thyroid were observed. Perchlorate levels in amniotic fluid were higher than the fetus serum levels in all dose groups.

^{125}I radioactivity in the dam GI contents was higher than the dam serum and ^{125}I radioactivity in the fetal gastric tract higher than the fetal serum. ^{125}I uptake in the mammary tissue was inhibited by perchlorate.

Dose-dependent increases in serum TSH and decreases in T_4 and T_3 in the GD20 and PND5 dams were observed. This dose-dependent trend of increased TSH and decreased thyroid hormones was also seen in the fetus.

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